PHYSIOLOGICAL RESPONSES TO LIVE E. COLI ORGANISMS

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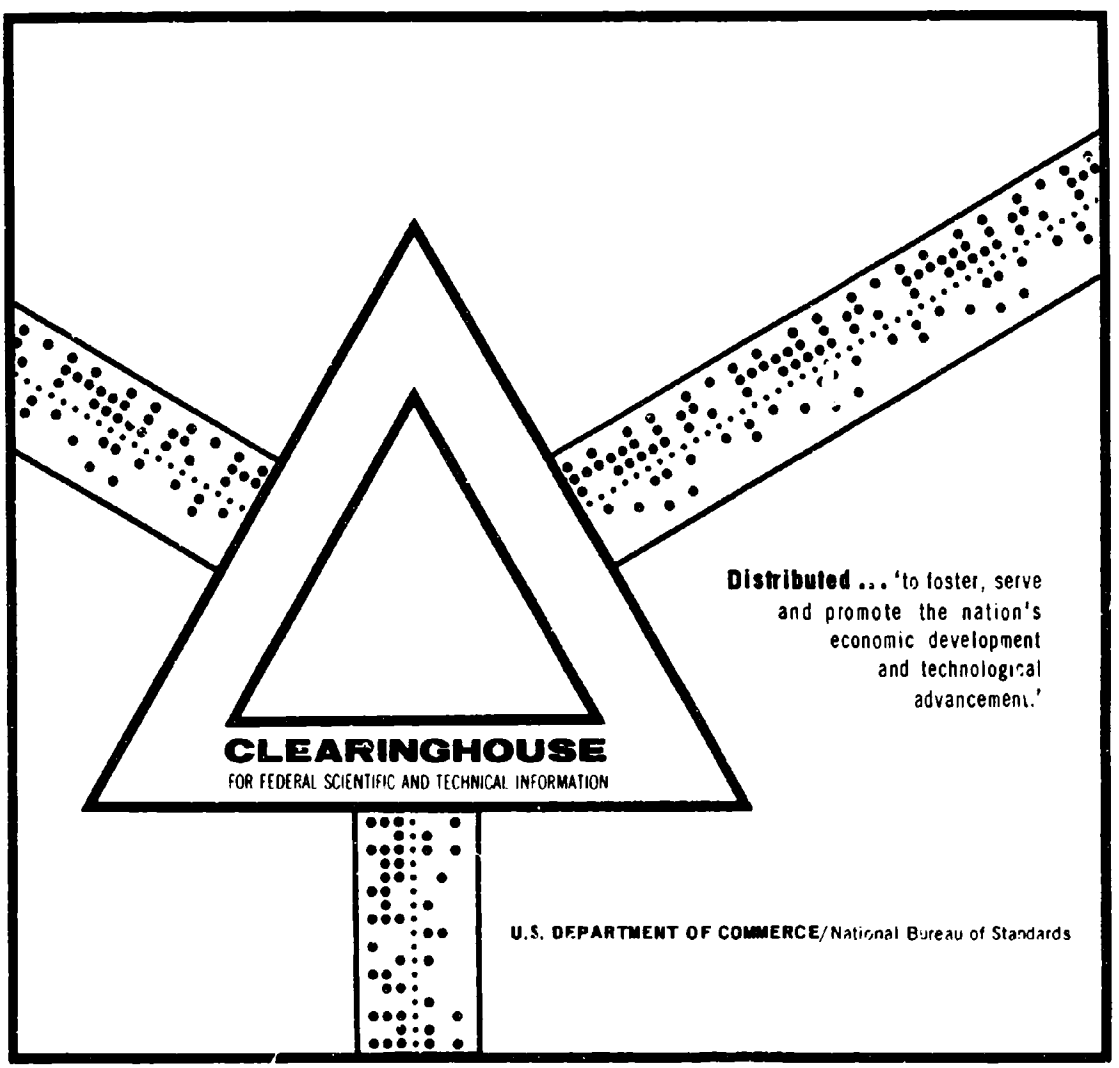

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UNIVERSITY OF OKLAHOMA MEDICAL CENTER

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MEDICAL CENTER RESEARCH AND DEVELOPMENT OFFICE
OF THE UNIVERSITY OF OKLAHOMA FOUNDATION, INC.
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Gram-negative septic shock has become a serious problem in clinical medicine. It is characterized by hypotension and inadequate blood flow and may occur with almost every specific infectious process. Endotoxin shock, or experimental septic shock, has been studied extensively in animals in this laboratory (1-5). Use of live E. coli organisms instead of endotoxin, to precipitate shock in an animal model, appear to offer significant advantages to an understanding of the underlying mechanisms of septic shock in man (4). In contrast to findings in dogs given endotoxin (1-3), results with live E. coli organism injection suggest that factors other than hepatic pooling may be responsible for the decrease in cardiac output and systemic arterial hypotension.

There is a growing concern regarding the failure to develop an effective treatment of septic shock in clinical medicine. Successful therapy would be immensely supported by an understanding of the basic mechanisms of the shock state. The development of a more realistic animal model would greatly aid in elucidating clinical problems observed in the human patient, and such is the goal of our research. Preliminary research in this laboratory suggested some differences between endotoxin shock and shock produced by injection of live E. coli organisms in dogs. A primary purpose of our research has been to determine the effects of intravenous injections of living E. coli organisms in dogs and monkeys and compare them with responses produced by endotoxin. Hemodynamic changes, pathologic alterations, and metabolic abnormalities have been evaluated in animals receiving lethal and sublethal injections of live E. coli organisms and comparable dosages of purified endotoxin. A major purpose of these experiments is to more closely

approach the clinical entity of septic shock by studying critical hemodynamic and metabolic parameters in dogs and monkeys.

An informative editorial review by Walsbren entitled, "Gram-Negative Shock and Endotoxin Shock" is replete with pertinent questions regarding the selection of a proper experimental model for the study of clinical septic shock. In the editorial, Walsbren reviews the historic development of concepts regarding the clinical picture of gram-negative bacteremia in man and of endotoxin shock in animals. Walsbren poses the question: "Is the shock-like clinical picture that results from bacteremia of gram-negative bacilli due to endotoxin?"

Borden and Hall (7) and Braude and others (8) were the first to suggest that gram-negative shock is caused by endotoxin. Walsbren, on the other hand, believed that the history of endotoxin research hardly seems to justify the view that endotoxin is the cause of gram-negative shock. Because of the assumption that the endotoxin molecule is primarily responsible for pathological effects observed during gram-negative bacteremic shock (9), animal studies have been carried out with purified endotoxin. Several years ago it occurred to us that the use of injected live *E. coli* organisms instead of endotoxin would be a procedure which would more closely approximate the clinical shock entity. Currently, there are several experimental reports describing results from live *E. coli* organism studies in animals (4,5,10,11). To date, the most significant difference pertaining to injection of endotoxin and live *E. coli* organisms has been the observation that following a lethal injection of live organisms marked renal hyperemia is observed in approximately half of the animals injected. This event has not been reported in endotoxin-

treated animals and appears to reveal a subtle but a possibly important difference between the live *E. coli* and endotoxin shock models. The question, therefore, of a proper experimental animal shock model for the study of bacteremic shock in man remains as yet unanswered.

The *E. coli* organism used in the experiments in this laboratory is an enteropathic Dunwald strain of *E. coli* typed as 0125:B15 (Canioni). Cultures are maintained on tryptic soy agar at 5° C and are transferred weekly. Considering the production of the organisms, tryptic soy broth is inoculated from stock culture and incubated 4 to 6 hours at 37° C, until growth is visible. Tryptic soy agar slants are inoculated from a tryptic soy broth and incubated for 18 hours at 37° C. In preparing the organism for injection, cells are transferred from tryptic soy agar slants by washing with isotonic saline. The culture is then centrifuged and resuspended in isotonic saline. The concentration of organisms (as determined by the percent transmittance in a BL Spectronic 20) was established which is lethal in 60-80% of animals receiving a 1 ml solution of inoculum per kg body weight. The concentration of a suspension of organisms was adjusted to the desired transmittance by dilution with isotonic saline. The viable count of the inoculum was determined by colony counts of serial dilutions in pour plates.

Emerson and Kelly (10) found that injection of living *E. coli* organisms led to the development of a shock-like state in most dogs tested. Typically, these organisms caused a gradual fall in mean arterial blood pressure, initial bradycardia followed by tachycardia, acidosis, hemoconcentration, and pathological lesions of the small bowel. These events culminated in death in the majority of animals. The gradual fall of arterial pressure paralleled the

decrease of viable *E. coli* cells in the blood, which suggested that destruction of the live organisms was directly linked to the fall of arterial pressure and pathological changes. These authors found that pathological changes observed in dogs given living *E. coli* cells resembled changes produced by *E. coli* endotoxin in earlier studies. They observed no important differences between dogs stressed with *E. coli* endotoxin and live *E. coli* organisms. Their data supported the concept that the endotoxin molecule was a major factor in the pathogenesis of gram-negative bacteremic shock.

Experiments have been carried out in this laboratory (4) comparing the canine responses to live *E. coli* organisms and *E. coli* endotoxin. Differences were seen in the canine responses to live *E. coli* organisms, and these observations correlated well with those changes observed in septic shock in man. Systemic hypotension became progressively more intensified after the injection of live organisms and did not appear to be associated with portal hypertension. Factors other than live pooling may therefore be considered important in accounting for the decrease in venous return observed in these experiments. Only slight early changes in portal pressures were seen at 2 minutes following injection; after 15-60 minutes, systemic arterial pressure decreased steadily from a mean of 124 to 76 mm Hg, while portal venous pressure was unchanged. However, by contrast, in all instances after endotoxin injection, there was a positive correlation between increase in portal venous pressure and decrease in systemic arterial pressure. This quantitative relationship did not prevail after administration of live organisms, since a marked decrease in systemic arterial pressure was usually seen to occur with only minimal changes in portal venous pressure. Venous

return decreased to the same low levels after both endotoxin and live organisms, but since portal hypertension was not a significant feature in shock induced with *E. coli* organisms, pooling presumably occurred in extrasplanchnic regions. A continued and persistent decrease in venous return and total peripheral resistance after injection of live organisms suggested that peripheral pooling may be generalized, with no specific sites implicated. The decrease in vascular resistance has also been reported in septic shock in recent clinical reports (13,14). It is particularly significant in the study reported by Hinshaw and others (4) that peripheral resistance decreased in spite of a decrease in distending pressure. Since resistance would be expected to increase passively as a function of a marked decrease in perfusion pressure, results suggested that either reflex mechanisms increasing resistance were defective or that active vasodilatation was occurring. The results of this study (4) appear to be at variance with recent clinical reports (13,14), since cardiac output did not increase in any of the dogs studied in the former series. This observation of our failure to approach the clinical entity is not understood. However, fever and an increased metabolic rate associated with sepsis were noted only occasionally in the study with dogs. The use of barbiturate anesthesia may have prevented a pyretic action of the organisms by depressing central nervous centers, thereby preventing an increase in cardiac output secondary to an increase in metabolic rate.

Results from renal studies carried out in this laboratory (4) were of particular interest. It was clear that two distinct types of responses were elicited by injection of live organisms: In the first type, renal hyperemia lasting for one hour developed progressively after an initial brief period

of renal vasoconstriction. An increase in urine flow paralleled the elevated renal blood flow which returned to control values. Renal vascular resistance progressively decreased during the first hour and remained constant thereafter, even though arterial pressure persisted at shock levels. Earlier studies carried out in our laboratory in dogs showed that renal blood flow increased significantly for one hour after small amounts of endotoxin were injected. Renal blood flow was also found to be elevated after larger sublethal injections of endotoxin if arterial pressure was not permitted to decrease to shock levels. Other investigators have noted an increase in renal blood flow after an initial phase of vasoconstriction when a pyrogen was injected into patients. No known clinical report has revealed renal hyperemia in septicemia, but this is not surprising since measurements of renal hemodynamics have presumably not been carried out during the initial phase of shock or the pre-shock period in patients. The second type of renal response was characterized by a progressive decrease in renal blood flow and urine flow, which led to anuria after injection of live *E. coli* organisms. Renal vascular resistance was elevated for one hour following injection, while systemic arterial pressure remained low in the shock range and renal blood flow progressively fell. Both oliguria and anuria are commonly seen in patients in septic shock. The decrease in renal function in patients in septic shock has been considered due to renal vasoconstriction and the resultant reduction in renal blood flow subsequent to a decrease in cardiac output. Results from the two types of renal responses in the present reported series suggest that renal hyperemia may not occur if systemic arterial pressure decreases to shock levels. It is possible

that forces tending to dilate renal arterioles are masked because of sympathoadrenal discharge subsequent to a decrease in arterial pressure.

The circulatory responses to an intravenous injection of viable washed *E. coli* organisms were studied in the dog and monkey by Thomas and others (11). They reported that the intravenous injection of gram-negative microorganisms elicits shock with a hemodynamic pattern identical to that following injection of purified endotoxin. They further reported that the circulatory response to the injection of viable *E. coli* was not initiated by free endotoxin in the bacterial suspension. They concluded that the similarity of the hemodynamic response elicited by gram-negative organisms to that initiated by purified endotoxin supports the use of the endotoxin shock model as a means by deriving meaningful information in the study of clinical septic shock. However, in their studies in the monkey, the small rise in portal pressure did not appear to cause the drop in arterial pressure. Although their results were assumed due to the presence of viable washed *E. coli* cells rather than endotoxin, within 30-90 seconds following injection, precipitous decreases in systemic arterial pressure and cardiac output occurred. This response is very similar to that obtained by massive injections of endotoxin and is not in agreement with studies carried out in this laboratory with live *E. coli* organisms (4). The results of the studies by Thomas and others (11) pertaining to discrepancies with experiments carried out in this laboratory (4) are not understood. It may be that the presence of a contaminant in their solutions resulted in the rapid increase in portal venous pressure followed by a subsequent marked drop in arterial pressure within 60-90 seconds following injection of living

cells. Work in this laboratory utilizing live organisms clearly shows a period of time following injection during which no hemodynamic event takes place. This time may vary between 10 and 30 minutes, and it would appear that the response to *E. coli* organisms depends on events that occur more slowly in time than following endotoxin injection. For this reason, the use of live *E. coli* organisms in an animal shock model would appear to more closely simulate the pre- and post-shock periods in the patient.

Septicemia and the administration of endotoxin may have different roles in the production of shock. Hemodynamic, respiratory, and metabolic effects of live organisms were compared with endotoxin injections in Rhesus monkeys by Guenter and others (5). The most striking differences between the group given endotoxin and *E. coli* organisms was the time of onset of measurable changes. Animals administered endotoxin developed hypotension, decreased cardiac output, and ventilatory changes much earlier than those given *E. coli* organisms. The severe hypoxia observed within five minutes of infusion of endotoxin was not observed in any of the animals given *E. coli* organisms. However, all animals in these two groups demonstrated decreased arterial PO_2 or increased alveolar-arterial oxygen gradients at some time during the course of the study.

Lungs from monkeys treated with endotoxin or live organisms have been studied by light and electron microscopy (12). Fifteen minutes after injection, engorgement of polymorphonuclear leukocytes within the pulmonary capillaries were observed. Edema of the perivascular space was noted in all lung tissues examined. Fragmentation of polymorphonuclear leukocytes was observed within four hours following injection. Endothelial cellular membranes appeared indistinct only at sites where polymorphonuclear leukocytes

were sticking. A widespread but focal perivascular edema observed in these studies could explain some of the physiological functional derangements seen in shocked monkeys. No differences were observed in lung tissue changes produced by endotoxin or *E. coli* organism injections.

From analysis of the past findings, what may be said about the benefits of the live *E. coli* organism shock model over the animal shock model produced by injection of endotoxin? Any answer would be inconclusive at this time due to lack of experimental data. It may be that the primary components involved in eliciting the hemodynamic, metabolic, and lethality characteristics are similar after both endotoxin and live *E. coli* organism injections. However, caution should be exercised in this regard. For example, in the dog, the response to endotoxin can be simulated very closely by injections of either massive amounts of histamine or a histamine liberator, 48-80; and yet, it is quite clear that histamine is only one of many substances involved in the production of shock after endotoxin. There may be subtle differences in the shock model as produced by endotoxin or live organism injection, and these differences may be important in regard to their clinical application. As a further point of interest, it has been calculated that endotoxin makes up about $\frac{1}{2}$ of 1% of the net weight of the live organism. Studies carried out in this laboratory have shown that 10^9 organisms/kg dog weight produce an approximate LD_{80}. However, a major discrepancy may be noted from the observation that 2 mg/kg of the so-called "purified" endotoxin is required to produce an equivalent LD_{80} in dogs. From the previous statement, it would therefore appear that approximately 400 mg/kg of live organisms would be required to produce the equivalent of the amount of endotoxin needed to produce a lethality effect equal in both instances. It

would therefore seem evident that either purified endotoxin has a large amount of impurities or that there is some toxic component contained within the live *E. coli* organism which produces the lethality characteristics on a basis different from endotoxin itself. A major difference observed between the response of a dog to endotoxin or live organisms is the renal response in which marked renal hyperemia may be observed in half of the animals receiving the organisms and has never been reported in animals receiving a lethal injection of endotoxin. An explanation for this observation is that some intermediate step elicited in the response to live organisms brings about renal vasocilatation on a presumably humoral basis. Renal hyperemia in the pre-shock phase of septic shock in man may be suspected on the basis that this phase of shock, or "pre-shock" in man, is characterized by an increase in skin temperature, tachycardia, and elevated temperature; and when a small sublethal injection of endotoxin is given, renal hyperemia is observed in patients. Therefore, it would be reasonable that following the injection of live *E. coli* organisms, there would be a period comparable to the "pre-shock" phase in man. The final point which must be evaluated is that the use of the live *E. coli* shock model may produce a more efficient and relevant clinical shock model in that the delayed period following the injection of organisms would be more comparable to that seen in man and would permit a more effective approach to therapy in the animal shock model. Future research will be required to elucidate similarities and differences between the models and provide the basis for a decision in regard to a more clinically relevant shock model for the study of septic shock in man.

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13. ABSTRACT

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